PROTOCOL TO PROMOTER ACTIVITY TEST UNDER CARBON STARVING

1. Make a pre-inoculation of DH5a :: puspA:GFP / / pJ23101 of 15 mL containing 15 µL of 35mg/mL cloramphenicol. (1 tube for each medium: LacBlue and LB). Incubate overnight at 37°C under 200 rpm.

2. 1mL of each medium should be collected to be used as test. Centrifuge at 4000rpm for 10 min. After, add 5 mL of medium LacBlue in the falcon previously containing LB and ressuspend cells in ice bath carefully. Return cells to shaker for 4h at 37°C under 200rpm. Keep falcons with ressuspended cells in new LB to comparison.

3. Recover aliquots of 100 uL per hour. Centrifuge at 5000rpm for 5 min and freeze the pellet.

4. Proceed protein extraction, as suggested by our protocol, by physical lysis using ultrasound. As provided by protocol "**PROTOCOL TO PRODUCTION OF [6xHis] - GFP**" (Steps 5 to 8).

5. Make following SDS-PAGE:

   Promoter J23101

   M LacBlueON 0h 1h 2h 3h 4h Soluble Insoluble

   Promoter UspA

   M LacBlueON 0h 1h 2h 3h 4h Soluble Insoluble

6. Test fluorescence of samples containing J23101 (J) and UspA (U) at final of experiment in a Black Elisa plate in Viktor (Perkin Elmer) fluorimeter.

   1 2 3 4 5 6 7 8 9 10 11 12

   B1 B2 B3 J1 J2 J3 J4 U1 U2 U3 U4 X **A (Medium LB)**

   B1 B2 B3 J1 J2 J3 J4 U1 U2 U3 U4 X **B (Medium BLUE)**

X X X X X X X X X X X X **C**

X X X X X X X X X X X X **D**

X X X X X X X X X X X X **E**

X X X X X X X X X X X X **F**

X X X X X X X X X X X X **G**

X X X X X X X X X X X X **H**

SOLUTIONS RECIPES AND BUFFERS

Lysis Buffer (1L):

Tris – 1,21g

NaH_2PO4 – 6,89g

NaCl – 5,84g

Adjust pH to 8.0, bring volume to 1L. Sterilize by autoclaving at 121°C / 15 min. Store at 4°C.

BLUE Medium (1L)

Bromothimol Blue – 0.08g

Ammonium Sulfate – 2g

Monobasic Potassium Phosphate – 13.6g

LB (Lysogenic Broth) – 1 mL of a solution 25g/1L

Cloramphenicol – 1 mL (35 mg/mL)

Sterilize by filtration at 0.22um seringe filter. Store at 4°C.